Transgenic mouse models of Alzheimer’s disease
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Introduction
Progress in the development of transgenic mouse models of Alzheimer’s Disease (AD) has come largely from the recent advances in the molecular genetics of the disease. Such studies have identified mutations in four different genes which are involved in early onset familial AD (FAD) [1-3]. They are the β-amyloid precursor protein (APP) gene on chromosome 21, the presenilin (PS)1 gene on chromosome 14, the PS2 gene on chromosome 1 and the apolipoprotein E (APOE) gene on chromosome 19. At least five missense mutations in the APP gene within or near the amyloid β-protein (Aβ) sequence have been identified leading to early-onset FAD and cerebral hemorrhage in several unrelated families. Three single-point mutations in exon 17 of APP at amino acid 717 (C terminus of Aβ) have been identified, with the valine residue substituted by either isoleucine, glycine or phenylalanine. In addition, a double-point mutation in the APP gene has been found in a large Swedish family with early onset (at about 55 years) FAD. The mutation consists of a lysine to asparagine and a methionine to leucine amino acid change at codons 670 and 671 respectively (Lys-670Asn, Met-671Leu) at the N-terminus of Aβ.

Mutations in the PS1 gene accounts for the majority (approximately 20–50%) of early onset (at about 28–50 years) FAD cases and about 2–4% (or less) of all AD cases. At least 42 missense mutations and a splice site mutation have been identified so far. Two mutations have been found in the PS2 gene, Asn-141Ile and Met-239Val, in Volgan German families with onset ages of about 40–75 years. The APOE ε4 allele is a susceptibility marker with incomplete penetrance, conferring a higher risk of developing AD at an earlier age.

The plasma, fibroblasts and platelets from patients with APP or PS1 mutations all show increased levels of Aβ1-42(3), which is deposited much earlier than Aβ1-40 in the brains of AD and Down’s syndrome patients and is potentially more amyloidogenic [4]. This evidence gives support to the current concept that the Aβ peptide is toxic to neurons and its increased generation from misprocessing of APP may be a major pathogenic mechanism. However, much evidence indicates that synapse loss, neuronal loss and paired helical filaments (PHFs) correlate better with clinical dementia than Aβ plaque load and distribution. Whether the early deposition of Aβ1-42(3), which may act as a nidus for Aβ1-40 accumulation, is the primary cause of AD...
remains an open and crucial question. It does not preclude the likelihood that the deposition of \(\alpha\beta_{1-42(3)}\) is secondary to a more fundamental pathogenetic mechanism that may interfere with the physiological functions of the genes described above.

With this background knowledge in the molecular genetics of AD several groups have generated mouse models of AD by testing the mechanisms of \(\alpha\beta\) deposition in transgenic mice by promoter-driven overexpression of human APP or PS mutant sequences. The ultimate aim is to determine if mice with \(\alpha\beta\) deposits in the form of senile plaques may also show sequelae of other neuropathological changes characteristic of AD such as reactive gliosis, synaptic loss, neuronal loss, dystrophic neurites, neurofibrillary tangles and learning and memory deficits. The other strategy that has been adopted is to generate mice with null mutations (knock-outs) of the mouse homologues of human APP, PS1 or APOE genes, the functions of which are not fully established. The neuropathological and phenotypic characteristics of such mice undoubtedly provide important clues to the biological functions of these molecules and their roles in AD.

**Transgenic APP or PS1 mice**

The transgenic mouse models that have attracted much attention are those which greatly over-express human APP or PS1 mutant sequences leading to genuine cerebral amyloidosis. These are: (i) the Athena Neurosciences and Exemplar Corporation transgenic mouse containing the transgene hAPP\(FAD\) valine to phenylalanine mutation and APP introns 6–8, enabling the production of the 695, 717 and 770 isoforms of APP, driven by platelet-derived growth factor-\(\beta\) (PDGF-\(\beta\)). Heterozygous mice showed more than 10 times more human APP than endogenous or human AD brain [5]; (ii) the Mayo Clinic mouse overexpressing the hAPP Swedish mutation (Lys-670Asn, Met-671Leu) driven by a hamster prion protein cosmid vector in which the level of hAPP overexpression was 3–5 times that of endogenous APP [6]; (iii) the two transgenic mice described by Novartis, namely one over-expressing the hAPP Swedish mutation combined with the hAPP valine to isoleucine mutation driven by the Thy-1 promoter producing a 2-fold over-expression and the other over-expressing the hAPP Swedish mutation alone driven by the Thy-1 promoter and producing about 7-fold over-expression of hAPP [7]. In general, the data indicate that in all of the above mice, the \(\alpha\beta\) deposition was age-dependent and region-specific and depended on the level of over-expression and specific mutations. Dense amyloid plaques were seen in the cortex, hippocampus, subiculum and presubiculum, were readily detected with thioflavin-S fluorescence and typically could also be labelled with Congo red to give the characteristic apple-green birefringence of classic amyloid. In the vicinity of plaques graded neurodegenerative changes were seen such as reactive astrocytosis and gliosis, dystrophic neurites and reductions in synaptophysin and microtubule-associated protein-2 (MAP-2) in the hippocampus indicating a loss of presynaptic terminal and dendrites. In addition, in the Novartis mice congophilic senile plaques were found to be immunoreactive for hyperphosphorylated tau, reminiscent of early tau pathology. However, neurofibrillary tangles have not been found in the brains of any of the above described transgenic mice. Ultrastructural studies done on the Athena mouse showed intracellular amyloid fibrils and some neurons displayed intranuclear inclusions, suggesting that apoptotic-like changes may take place in neural cells. All of these transgenic mice show robust deposition of \(\alpha\beta_{1-42(3)}\) as well as \(\alpha\beta_{1-40}\). In the Athena mouse \(\alpha\beta_{1-42(3)}\) was the main constituent (97%) of the amyloid in the 12-month-old heterozygotes. However, detailed stereological analysis of neurons in the entorhinal cortex (EC), including layer II (which is the first to degenerate in AD) in 18-month-old heterozygous mice and in which 20–50% of the entorhinal cortex contained \(\alpha\beta\) immunoreactivity, did not reveal any significant cell loss despite the heavy amyloid burden [8]. Significant cell loss was also not found in the Mayo mouse. However, even in the absence of demonstrable morphological deficits, this mouse showed impaired learning and memory by 9–10 months of age although not at 3 months of age [6]. In addition, electrophysiological studies showed normal synaptic activity and plasticity in animals <8 months old but impairment in older (>10 months old) animals [9].

In addition to transgenic hAPP mice, mice overexpressing hPS1 mutant sequences have been developed. The mutant hPS1 (but not wild-type hPS1) transgenic mice showed increased brain levels of \(\alpha\beta_{1-42(3)}\) by ELISA although no \(\beta\)-amyloid deposits have so far been detected [10]. Moreover, there are no reports of learning
and memory deficits in these mice. However, an accelerated AD-like phenotype has been observed in transgenic mice carrying both the hAPP Swedish mutation (Lys-670Asn, Met-671Leu) and hPS1 (Met-146Leu) transgene [11]. The doubly transgenic progeny develop large numbers of fibrillary Aβ deposits in the cerebral cortex and hippocampus far earlier (at 26 weeks, although one 16-week-old double transgenic showed deposits) than singly transgenic APP littermates. In the period preceding the overt Aβ deposition, the doubly transgenic mice showed a 41% increase in brain levels of Aβ1-42. Both the singly and doubly transgenic mice showed behavioural deficits (spontaneous alternation performance in the Y-maze) at about 12 weeks of age, i.e. much earlier than extracellularly deposited Aβ. The results suggest that PS1 and APP mutations may be synergistic, possibly leading to enhanced Aβ production. The finding that a behavioural change precedes the overt deposition of Aβ by many weeks may indicate that the pathogenic event precedes plaque formation. This may be related to intracellular Aβ accumulation, or may be independent of Aβ or may be related to the expression of the transgene itself. It will be crucial to determine if the doubly transgenic mice which show high levels of Aβ1-42 develop tangles or significant neuronal loss with age which may have a greater influence on learning and memory.

The generation of high levels of hAPP or hPS1 transgene expression and the robust deposition of Aβ, especially the Aβ1-42 species in the brains of the transgenic mice is definitely a significant step forward in the attempts to generate a mouse model of AD. However, the robust deposition of Aβ in the absence of significant neurodegenerative changes, especially the loss of neurons, may question the Aβ neurotoxicity hypothesis. Over-expression of the APP or PS1 mutation may itself produce limited neurodegenerative changes in the brains of mice independent of Aβ. It is likely that the deposition of Aβ is secondary to a more fundamental pathogenic mechanism occurring in AD brain. If AD mutations increase the levels of Aβ they must also interfere with the normal processing of APP or PS1 and compromise the physiological function of these molecules. Thus, the precise biological function of these genes needs to be determined if we are to understand their roles in the pathogenic mechanisms that lead to AD.

Mice with null-mutations of these genes have provided some answers to these questions.

**Mice with null-mutations of APP or PS1 genes**

Mice with a complete deletion of endogenous APP have been reported to show a significant impairment in long-term potentiation (LTP), age-related cognitive deficits and a pronounced decrease in the density of the presynaptic vesicle marker proteins, synapsin and synaptophysin and the dendritic marker, MAP-2 in several brain areas, but most predominantly in the cortex and hippocampus (G. R. Dawson, G. R. Seabrook, H. Zheng, D. W. Smith, S. Graham, G. O'Dowd, B. J. Bowey, S. Boyce, M. E. Trumbauer, H. Y. Chen, L. H. T. Van der Ploeg and D. J. S. Sirinathsinghji, unpublished work). Thus, the observed behavioural and LTP deficits may be related to alterations in the patterns of functional connectivity within the cortex and hippocampus. This concept is strongly supported by data showing that synaptic APP increases with learning capacity in rats [13]. Intracerebroventricular injections of APP also increase presynaptic synaptophysin terminal density and memory retention in rats [14] and protect the central nervous system against ischaemic damage [15]. Interestingly, wild-type hAPP751 offered protection while a hAPP valine to isoleucine mutation did not, indicating that the mutation may have compromised the neuroprotective effects of APP [15]. APP may have a trophic role and may also be critically involved in the maintenance of neuronal homoeostasis and synaptic functions during ageing; an impairment in APP metabolism at the synaptic junctions may thus contribute to the neuronal dysfunction seen in AD. Early onset AD involves synaptic loss as an early event and a loss of synaptic markers and synapses correlates better with dementia in AD than with either plaques or neurofibrillary tangles. The factors that alter the balance between the neurotropic and detrimental effects of APP are crucial to the elucidation of the pathogenic mechanisms in AD.

The PS-1 deletion in the mouse is a lethal mutation: homozygous PS-1-null mice do not survive to term [16,17]. Embryos show severe abnormalities in the developing axial skeleton and spinal ganglia, phenotypes traced to defects in somite segmentation and differentiation. Such defects are not seen in the heterozygotes. All PS-1-null embryos after E11.5 show haemorrhages limited to the brain and/or spinal cord.

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(haemorrhages present beneath the leptomeninges, in the ventricles and in the neural parenchyma, e.g. in the cortex, thalamus and striatum). In addition, PS1-null embryos show an impairment in neurogenesis and a severe loss of neural progenitor cells and neurons in the ventral-lateral region of the ventricular zone and in the subcortical region of the temporal lobe respectively. These data indicate that PS1 may play a critical role in embryonic development, formation of the axial skeleton and in normal neurogenesis and survival of progenitor cells and neurons in discrete brain areas. The somite segmentation defects seen in the PS1-null embryos are similar to those seen in embryos with functionally inactivated Notch 1 orDll1 (delta-like gene 1), a vertebrate Notch ligand allele. Some data suggest that PS1 may regulate the expression and function of Notch and Dll 1 in establishing somite borders and segmentation [16]. The cerebral haemorrhages seen in the PS1-null embryos are also seen in mice with a functionally ablated Dll 1 gene. This suggests that these genes may be involved in the development of the cerebrovasculature through facilitation of Notch signalling pathways. Indeed, the presenilins have about 50% and 25% homology with the Caenorhabditis elegans protein, SEL-12 and SPE-4 respectively. SEL-12 facilitates signalling via the LIN-12/Notch family of receptors which are involved in cell fate and determination. Wild-type PS1 and PS2 cDNAs can complement SEL-12 function effectively, while cDNAs containing PS1 and PS2 mutations have a reduced ability to rescue SEL-12 mutations, indicating that mutations may result in a loss of function by lowering them to a level below that required to maintain optimal function (haploinsufficiency phenotype). The SPE-4 protein in C. elegans plays a role in the transport of proteins to spermatids during spermatogenesis and this process is abolished by mutations in SPE-4 [1,2]. Thus, PS1 may also be involved in the cellular transport, trafficking and/or processing of membrane-bound and secretory proteins, e.g. APP. It is also likely that PS1 mutations may have a direct effect on neuronal survival in the adult brain, relevant to the loss seen in AD. The data from the PS1-null mice have highlighted the complex interaction between PS1 and Notch, although we do not understand the exact mechanism or sites of action of PS1 in the Notch signalling pathway and how critical this is for AD. However, 10 different mutations in the Notch 3 gene localized on chromosome 19 have been found in 14 unrelated individuals suffering from a syndrome characterized by stroke and dementia, called cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) [18]. Characteristic features of CADASIL include recurrent subcortical ischaemic strokes, vascular dementia, craniofacial paralysis, mood disorders with severe depression and migraine. It is clear that disruption of the PS1/Notch pathways may be a key factor in the mechanisms that lead to dementia in the adult.

Conclusion
There is no doubt that in the last 3–4 years there have been striking advances in attempts to develop an appropriate mouse model of AD. The transgenic mice that have been generated to over-express human APP mutant sequences leading to amyloidosis in the brain, allowed the Aβ neurotoxic hypothesis to be tested. In these mice there is evidence for graded neurodegenerative changes (mainly in the vicinity of the plaques) but one may ask if these changes are mainly due to the neurotoxic effects of Aβ or simply due to the large accumulation of Aβ deposits in brain parenchyma. Despite the robust production and deposition of Aβ1-42, there is no evidence for frank neuronal loss or synaptic loss anywhere in brain or along specific neuronal pathways. One possible reason for this is that the endogenous mouse APP or the promoter-driven increase in neurotrophic/neuroprotective APP fragments may prevent cell death. Transgenic mice with varying levels of human APP over-expression and bred into the APP-null background may be useful animals to address some of these issues. Recent strategies have also utilized the Cre-lox approach to target the APP gene by inserting an APP FAD mutation and humanizing the Aβ sequence [19]. In addition, a ‘hit and run’ gene targeting technique has been used for introducing three humanizing changes and two APP FAD mutations into the mouse genome [20]. Other strategies are already available for generating multiple lines of transgenic mice over-expressing two or more AD genes, e.g. PS1 and APP FAD in a normal mouse background [11] or in the null background. These strategies are being used mainly to test the neurotoxic potential of Aβ. It is feasible that Aβ may not be the primary event in the disease process and this could be tested by over-expressing the PS1 FAD...
mutation in the APP-null background. We also need to understand the roles of APOE and tau and their interactions with APP or PS1. Mice can now be generated over-expressing any combination of these genes. The next few years should yield even greater advances than we have so far witnessed. It is clear that a valid mouse model of AD shall be of immense value to delineate the key initiating neurodegenerative events and to develop rational therapeutic strategies to prevent or arrest the progression of the disease.

12 Reference deleted
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